Antimicrobial peptide gene expression in medication-related osteonecrosis of the jaw

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Abstract: Bisphosphonates and denosumab are commonly used antiresorptive therapies in patients with bone metastasis and osteoporosis. Medication-related osteonecrosis of the jaw (MRONJ) is a serious side effect of these drugs, and infection has been recognized as a contributing factor. Current therapeutic options for MRONJ show limited effectiveness, therefore necessitating novel treatment strategies. Bisphosphonates have recently been reported to induce the expression of antimicrobial peptides (AMPs), an inherent component of the immune system. Therefore, the aim of the present study was to investigate and compare the influence of the anti-RANKL antibody denosumab and bisphosphonates on the gene expression of selected AMPs: human α-defensin-1, human α-defensin-3, human β-defensin-1, and human β-defensin-3. Bone specimens were collected from patients with MRONJ who had been treated with bisphosphonates (n = 6) or denosumab (n = 6), and from healthy subjects (n = 6) with no history of treatment with bone metabolism-influencing drugs. Reverse transcription-quantitative polymerase chain reaction was used to quantify the expression levels of selected AMPs. Samples from patients treated with denosumab showed significantly higher mRNA expression of human α-defensin-3 and human β-defensin-3 than those from healthy subjects. This finding is similar to previously described upregulated expression of human defensins in patients with MRONJ after bisphosphonates treatment. This suggests that the elevated expression of defensins may be at least a part of the mechanism underlying the pathogenesis of osteonecrosis induced by antiresorptive therapies, which can serve as a new target for potential treatment of MRONJ.

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Original article

Antimicrobial peptide gene expression in medication-related osteonecrosis of the jaw

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ABSTRACT

Bisphosphonates and denosumab are commonly used antiresorptive therapies in patients with bone metastasis and osteoporosis. Medication-related osteonecrosis of the jaw (MRONJ) is a serious side effect of these drugs, and infection has been recognized as a contributing factor. Current therapeutic options for MRONJ show limited effectiveness, therefore necessitating novel treatment strategies. Bisphosphonates have recently been reported to induce the expression of antimicrobial peptides (AMPs), an inherent component of the immune system. Therefore, the aim of the present study was to investigate and compare the influence of the anti-RANKL antibody denosumab and bisphosphonates on the gene expression of selected AMPs: human α-defensin-1, human α-defensin-3, human β-defensin-1, and human β-defensin-3. Bone specimens were collected from patients with MRONJ who had been treated with bisphosphonates (n = 6) or denosumab (n = 6), and from healthy subjects (n = 6) with no history of treatment with bone metabolism-influencing drugs. Reverse transcription-quantitative polymerase chain reaction was used to quantify the expression levels of selected AMPs. Samples from patients treated with denosumab showed significantly higher mRNA expression of human α-defensin-3 and human β-defensin-3 than those from healthy subjects. This finding is similar to previously described upregulated expression of human defensins in patients with MRONJ after bisphosphonates treatment. This suggests that the elevated expression of defensins may be at least a part of the mechanism underlying the pathogenesis of osteonecrosis induced by antiresorptive therapies, which can serve as a new target for potential treatment of MRONJ.

1. Introduction

Antiresorptive drugs that influence bone metabolism, such as bisphosphonates and the receptor activator of nuclear factor κB ligand (RANKL) antibody denosumab, are important as treatment options for osteoporosis and as a supportive therapy for osseous metastatic malignancies, by regulating the resorption of bone [1]. However, since their introduction, the incidence of bisphosphonate-related osteonecrosis of the jaw (BRONJ) has increased steadily [2]. In 2010, Taylor et al. [3] reported the first case of osteonecrosis induced by anti-RANKL antibody (denosumab), with similar radiological, clinical, and histological characteristics as observed in BRONJ lesions. A comparative study in 2013 showed that the risk of development of osteonecrosis of the jaw was similar between patients treated with the bisphosphonate zoledronate and denosumab [4].

Considering these general findings, in 2014, the American Association of Oral and Maxillofacial Surgeons introduced the term "medication-related osteonecrosis of the jaw" (MRONJ), which clinically manifests as "exposed necrotic bone (or bone that can be probed through an intraoral or extraoral fistula) in the mandible or maxilla for at least 8 weeks' duration, with or without the presence of pain or infection, in a patient with no history of radiation therapy or obvious metastatic

Abbreviations: MRONJ, Medication-related osteonecrosis of the jaw; AMPs, antimicrobial peptides; BRONJ, bisphosphonate-related osteonecrosis of the jaw; RANKL, receptor activator of nuclear factor κB ligand; hAD, human α-defensin; hBD, human β-defensin.

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diseases in the jaws and under current or previous treatment with antiresorptive or antiangiogenic agents" [5]. Antiangiogenic drugs are used for tumor patients to interfere with various steps in angiogenesis, thus inhibiting tumor growth [6]. Currently, there are no globally accepted treatment options for MRONJ. Treatment strategies range from conservative non-surgical therapy to early surgical intervention. The extent of an intervention depends on disease stage, presented symptoms, and other factors such as the underlying morbidity and comorbidities [5]. Therefore, further research is needed to understand the underlying pathogenic mechanism contributing to MRONJ for the development of effective targeted therapies.

As key elements of innate immunity, human antimicrobial peptides (AMPs), such as defensins, play a critical role against microbial pathogens [7]. Furthermore, AMPs are involved in other biological functions such as apoptosis, wound healing, and immune modulation [7]. Additionally, AMPs are responsible for mediating surface immunity. To avoid bacterial invasion, the expression of AMPs on mucosal and dermal surfaces is increased to stabilize the epithelial barrier [8]. Therefore, protecting the bone from pathogenic flora is an important function of AMPs.

Human defensins are small cationic peptides with a large number of hydrophobic amino acids. Their interaction with the anionic membranes of gram-negative and gram-positive bacteria and encapsulated viral and fungal pathogens, facilitates the destruction of the cellular membranes and interferes with the intracellular functions of these microorganisms [9]. Among the numerous defensins discovered to date, human β-defensins (*hBD*s), especially *hBD-1* and *-3*, are known to have a broad-spectrum antimicrobial activity and constitutional similarities [10]. Human α-defensins (*hAD*s) have comparable functions that vary depending on the type of bacteria [7]. In addition, they show chemotactic activity and modulate inflammation by influencing the expression of cytokines and adhesion molecules [11]. In 2005, Warnke et al. detected the expression of *hBD-1* and *-3* in chronically diseased and healthy jawbones, thereby providing the first confirmation of the inherent immunological function of bone [12].

Elevated *hBD* levels have been detected in osteomyelitis of the jaw, and Stockmann et al. quantified their expression in MRONJ compared with expression in osteoradionecrosis and healthy jawbones (control) [13]. Their results confirmed that the jawbone of MRONJ patients expressed higher levels of beta-defensins when compared to healthy uninfected jawbones. Elevated human defensin levels indicate that defective defensin expression in bone might lead to inflammation and subsequently osteonecrosis of the jawbone [13].

Based on this background, the aim of the present study was to investigate and compare the effects of antiresorptive drugs on the gene expression of selected α- and β-defensins in jawbone samples from patients with MRONJ compared to that in healthy jawbones. We report increased mRNA expression of defensins mediated by denosumab treatment similar to that induced by bisphosphonate therapy in patients with MRONJ.

2. Materials and methods

2.1. Patients and control subjects

This prospective study, with the subsequent use of biological material, was conducted in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research. The cantonal ethics committee of Zurich (Approval No. F-42880-06-0) approved the study, and prior written informed consent was obtained from all individual participants for the study.

Bone samples of 12 patients diagnosed with MRONJ, six patients treated with bisphosphonates and six patients treated with denosumab, for more than 12 months, were used in the study. Patients who previously used antiresorptive drugs (both denosumab and bisphosphonates) and those with asymptomatic MRONJ not requiring a surgical intervention were excluded.

The samples were harvested during therapeutic necrosectomy of the maxilla and/or mandible (which are otherwise routinely discarded) and included the transitional zone between non-necrotic and necrotic bones.

Bone samples for the control group were harvested from six healthy individuals, with no history of bone metabolism-influencing drugs or serious diseases of the bone (e.g. osteoporosis). The samples were obtained during the osteotomy and/or extraction of their wisdom teeth.

2.2. RNA purification and reverse transcription

The total RNA was extracted and purified from the bone samples using a commercial RNA extraction kit (miRNeasy Mini Kit; QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity of the total RNA were determined by spectrophotometric measurements at 260 and 280 nm (NanoDrop 2000®; Thermo Fisher Scientific, Waltham, MA, USA). The extracted RNA samples were frozen and stored at - 80 °C until analysis.

The total RNA (400 ng) was reverse transcribed into cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The cDNA samples were diluted to 1/15 and stored at −20 °C (total volume 600 µL). SsoAdvanced™ PreAmp Supermix® (Bio-Rad) was used according to the manufacturer's protocol to provide unbiased preamplification, enabling to obtain more data from a limited sample size.

The cDNA was used as a template to determine the basal mRNA expression level of human *hAD-1*, *hAD-3, hBD-1*, and *hBD-3* in each of the 18 samples by qPCR. Patient samples were divided into three groups: Group 1 comprised the six samples from patients treated with denosumab, Group 2 comprised the six samples from patients treated with bisphosphonates, and Group 3 comprised the six samples from healthy subjects. The results of the two pathological sample groups (Groups 1 and 2) were independently compared with those of the healthy sample group (Group 3).

2.3. Quantitative polymerase chain reaction (qPCR)

PCRs were performed using 10 µL of diluted reverse transcriptase (RT) product per reaction according to the manufacturer's protocol (SsoAdvanced™ Universal SYBR® Green Supermix; Bio-Rad). The primers used in this study were purchased from Bio-Rad and are listed in Table 1. In addition to the selected defensins, the housekeeping gene *RPS18* was simultaneously amplified with each gene of interest as an internal reference. Amplification was conducted on the CFX Connect Real-Time PCR System (Bio-Rad).

2.4. Statistics

Relative gene expression levels were determined using the 2ΔΔCT method [14], and these data are reported in the form of boxplots using the ΔCT values (ΔCT = CT target gene - CT reference gene). *t*-test of the ΔCT values was used to determine differences in the expression profiles using XLSTAT-Excel Statistical Calculator (Microsoft, Redmond, WA,

Table 1
List of defensins and the reference gene tested by qPCR.

Gene/ Defensin	Name	Primer Provider	Primer ID (catalogue number)
hAD-1	Human α-defensin-1	Bio-Rad	qHsaCID0037384
hAD-3	Human α-defensin-3	Bio-Rad	qHsaCED0056797
hBD-1	Human β-defensin-1	Bio-Rad	qHsaCID0015106
hBD-3	Human β-defensin-3	Bio-Rad	qHsaCED0037167
RPS18	Ribosomal protein S 18	Bio-Rad	qHsaCED0037454

USA).

Results with a *p*-value of <0.05 were considered statistically significant.

3. Results

The average age at surgery was 71.9 ± 10.1 years in the MRONJ group (Table 2) and 24.8 ± 4.2 years in the control group (Table 3).

3.1. hAD-1

There was no significant difference in *hAD-1* mRNA expression between the healthy and pathological groups (Fig. 1). *hAD-1* mRNA expression in the healthy group was higher than that in the denosumab group but lower than that in the bisphosphonate group. These differences were not statistically significant. By dividing the pathological groups according to the treatment, bisphosphonates or denosumab, we noticed that there was a significant difference with respect to an increase in the mRNA expression of *hAD-1* in the bisphosphonate group compared with the denosumab group.

3.2. hAD-3

There was a significant increase in *hAD-3* mRNA expression in both pathological groups, denosumab and bisphosphonates, compared to the healthy controls (Fig. 1).

3.3. hBD-1

All samples showed low mRNA expression of *hBD-1* (Fig. 2). There was no significant difference in *hBD-1* mRNA expression among the three groups, with the lowest expression observed in the healthy group.

3.4. hBD-3

The mRNA expression of *hBD-3* in the healthy group was almost zero (Fig. 2) compared with that in the denosumab group, which was significantly increased. Compared with the healthy group, the mRNA expression of *hBD-3* in the bisphosphonate group was also increased, but with no significant difference.

4. Discussion

MRONJ is a devastating complication of antiresorptive therapies defined by the exposure of bone and accompanied by pain, swelling, and infection of soft tissues. Currently, there are no globally accepted treatment protocols for MRONJ, resulting in a high rate of recurrence and persistence of symptoms. In the present study, we determined and compared the effects of antiresorptive drugs (bisphosphonates and denosumab) on the gene expression of selected human α- and β-defensins in the jawbone samples harvested from patients with MRONJ and healthy individuals.

Table 3
Demographic data of the healthy individuals.

Sample	Sex	Age (years)	Location
1	f	31	Lower jaw
2	m	24	Upper jaw
3	f	23	Lower jaw
4	m	19	Lower jaw
5	f	28	Lower jaw
6	m	24	Upper jaw

AMPs are key elements in the innate immune system and provide the first line of defense in the skin against invading microbes [10]. In this context, Nomura et al. investigated the expression of innate immune response genes, including that of *hBD*s, in two of the most common chronic skin diseases: atopic dermatitis and psoriasis. Although AMPs were detected in small amounts in normal skin keratinocytes of healthy individuals, large amounts were found to be produced in response to stimulation with injury or inflammation, and the atopic dermatitis lesions had lower levels of *hBD* than psoriasis lesions [15]. In particular, the authors showed that the most effective transcriptional stimulation of *hBD-3* was achieved by a combination of tumor necrosis factor-α and interferon-γ, two cell-signaling proteins (cytokines) involved in systemic inflammation and the acute phase reaction [15]. These findings could explain the high *hBD-3* mRNA expression observed in the MRONJ samples in this study, given that our patients were in an advanced disease stage, accompanied by infection and requiring surgical intervention. Despite many theories explaining the etiopathogenesis of MRONJ, several studies have highlighted infection as a major and not just secondary event, which stimulates a chronic inflammatory immune response [16]. This response would cause the upregulation of cytokines to ultimately upregulate *hBD-3* expression with the progression of the infection [16].

Consistently, Warnke et al. confirmed that the expression of AMPs "appears to be upregulated in chronically infected osteoradionecrotic mandibular bone" [12].

hBD-1 and *-3* were first identified in the mineralized bone matrix and cytoplasm of osteocytes of chronically infected mandibular bone samples [12]. Although expressed in lower quantities, the expression levels of both molecules were equal in each of uninfected bone types, including those in the controls [12]. These findings may reflect upregulation of AMP expression due to chronic infection and are comparable with our results for *hBD-1* and *-3*.

Surgical intervention and extended antibiotic therapy are currently the leading therapeutic options for MRONJ. Zirk et al. reported that different antibiotic regimens and local submucosal infection affect the clinical course of patients suffering from MRONJ, particularly in advanced disease stages [17]. However, antimicrobial resistance among

Table 2
Demographic data of the patients.

Sample	Sex	Age (years)	Antiresorptive drug	Disposal	Location	Underlying Diagnosis
1	m	75	Denosumab	s.c.	Upper jaw	Cancer
2	f	75	Denosumab	s.c.	Lower jaw	Osteoporosis
3	m	64	Denosumab	s.c.	Lower jaw	Cancer
4	f	72	Denosumab	s.c.	Lower jaw	Osteoporosis and Cancer
5	m	72	Denosumab	s.c.	Upper jaw	Osteoporosis
6	f	70	Denosumab	s.c.	Lower jaw	Cancer
1	m	65	Bisphosphonate	i.v.	Lower jaw	Cancer
2	f	92	Bisphosphonate	i.v.	Lower jaw	Osteoporosis
3	f	65	Bisphosphonate	i.v.	Lower jaw	Osteoporosis
4	f	75	Bisphosphonate	i.v.	Lower jaw	Cancer
5	f	85	Bisphosphonate	i.v.	Upper jaw	Osteoporosis
6	m	53	Bisphosphonate	i.v.	Lower jaw	Cancer

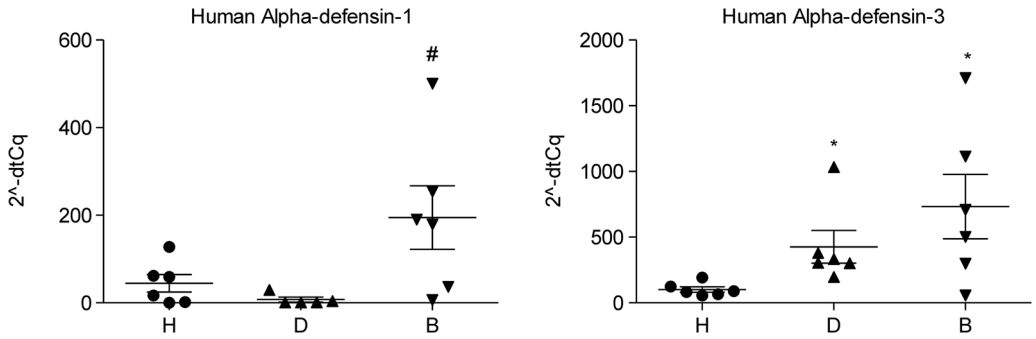

Fig. 1. Human α-defensin (*hAD-1* and *hAD-3*) mRNA expression determined by q-PCR. The graphs represent the expression in the healthy (**H**), denosumab (**D**), and bisphosphonate (**B**) groups normalized to *RPS18* expression using the $2^{-\Delta\Delta CT}$ method. * $p < 0.05$ vs. healthy; # $p < 0.05$ vs. denosumab.

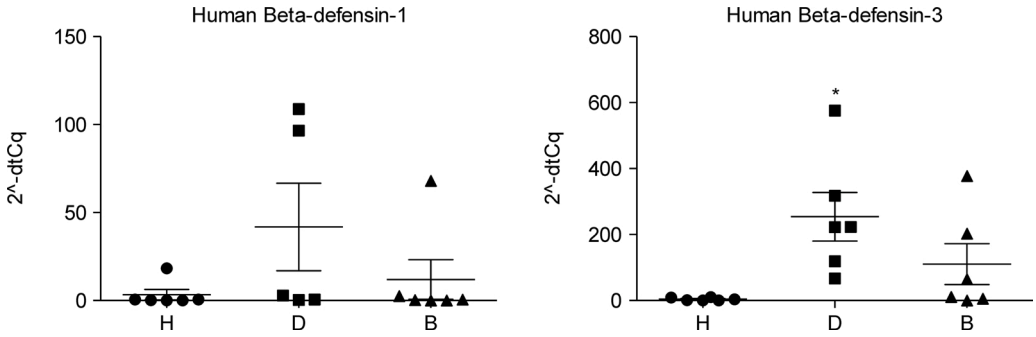

Fig. 2. Human β-defensin (*hBD-1* and *hBD-3*) mRNA expressions determined by q-PCR. The graphs represent the expression in the healthy (**H**), denosumab (**D**), and bisphosphonate (**B**) groups normalized to *RPS18* expression using the $2^{-\Delta\Delta CT}$ method. *$p < 0.05$ vs. healthy.

common bacterial pathogens is noticeably increasing, which is a substantial challenge in improving treatment outcomes [18]. In fact, the World Health Organization named antibiotic resistance as one of the "three most important public health threats of the 21st century" [19]. Given the decreasing potential of antibiotics as a therapy for MRONJ, defensins could be a potential and promising alternative for anti-infective compound development. The interaction of defensins with molecules of the microbial cell envelope that are directly accessible, such as lipopolysaccharides, can be highly specific [20]. Furthermore, defensins modulate the immune response regardless of the state of resistance of the individual pathogen [20]. Their broad spectra along with multifunctional characteristics make AMPs a promising constituent for developing therapeutic strategies not only against MRONJ but also against multiple other human diseases.

However, our study had some limitations. The study had a small number of patients, and there was no distinction between patients with osteoporosis and cancer. Furthermore, there was a large age difference between the control and patient groups.

Future research should focus on whether the administration of bisphosphonates and antiresorptive drugs impairs the antimicrobial activity of defensins. Furthermore, studies could focus on increased *hAD-3* mRNA expression in patients treated with antiresorptive drugs and elucidate why bisphosphonates and denosumab impair the expression of *hAD-1* in a significantly different way. In addition, these new findings related to the pathology and pathomechanism of MRONJ may serve as a model for the development of new therapies and prevention approaches.

5. Conclusions

In the present study, we demonstrated significantly increased mRNA expression of selected human defensins (*hAD-3* and *hBD-3*) in MRONJ-affected bones in patients treated with anti-RANKL antibody (denosumab). This upregulation was similar to that observed after treatment with bisphosphonates; although not to the same extent. These findings suggest that human defensins, especially *hAD-3* and *hBD-3*, could be a promising and innovative target for the prophylaxis and treatment of MRONJ.

CRediT authorship contribution statement

Yasmin Thiel: Data curation, Writing - original draft, Writing - review & editing. **Chafik Ghayor:** Data curation, Formal analysis, Methodology, Software, Validation, Visualization. **Daniel Lindhorst:** Funding acquisition, Project administration, Resources, Supervision. **Harald Essig:** Funding acquisition, Supervision. **Franz Weber:** Conceptualization, Investigation, Validation. **Martin Rücker:** Conceptualization, Investigation, Resources. **Paul Schumann:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing.

Declaration of Competing Interest

None.

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References

[1] W.C. Dougall, M. Chaisson, The RANK/RANKL/OPG triad in cancer-induced bone diseases, Cancer Metastasis Rev. 25 (2006) 541–549, https://doi.org/10.1007/s10555-006-9021-3.

[2] R.E. Marx, Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic, J. Oral Maxillofac. Surg. 61 (2003) 1115–1117, https://doi.org/10.1016/s0278-2391(03)00720-1.

[3] K.H. Taylor, L.S. Middlefell, K.D. Mizen, Osteonecrosis of the jaws induced by anti-RANK ligand therapy, Br. J. Oral Maxillofac. Surg. 48 (2010) 221–223, https://doi.org/10.1016/j.bjoms.2009.08.030.
[4] D. Henry, S. Vadhan-Raj, V. Hirsh, R. von Moos, V. Hungria, L. Costa, P.J. Woll, G. Scagliotti, G. Smith, A. Feng, S. Jun, R. Dansey, H. Yeh, Delaying skeletal-related events in a randomized phase 3 study of denosumab versus zoledronic acid in patients with advanced cancer: an analysis of data from patients with solid tumors, Support. Care Cancer 22 (2014) 679–687, https://doi.org/10.1007/s00520-013-2022-1.
[5] S.L. Ruggiero, T.B. Dodson, J. Fantasia, R. Goodday, T. Aghaloo, B. Mehrotra, F. O'Ryan, American Association of Oral and Maxillofacial Surgeons, American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw – 2014 update, J. Oral Maxillofac. Surg. 72 (2014) 1938–1956, https://doi.org/10.1016/j.joms.2014.04.031.
[6] P. Maione, A. Sgambato, F. Casaluce, P.C. Sacco, G. Santabarbara, A. Rossi, C. Gridelli, The role of the antiangiogenetic ramucirumab in the treatment of advanced non small cell lung cancer, Curr. Med. Chem. 24 (2017) 3–13, https://doi.org/10.2174/0929867324666161118125103.
[7] G. Wang, Human antimicrobial peptides and proteins, Pharmaceuticals (Basel) 7 (2014) 545–594, https://doi.org/10.3390/ph7050545.
[8] A. Dunsche, Y. Açil, R. Siebert, J. Harder, J.M. Schröder, S. Jepsen, Expression profile of human defensins and antimicrobial proteins in oral tissues, J. Oral Pathol. Med. 30 (2001) 154–158, https://doi.org/10.1034/j.1600-0714.2001.300305.x.
[9] R.L. Gallo, K.M. Huttner, Antimicrobial peptides: an emerging concept in cutaneous biology, J. Invest. Dermatol. 111 (1998) 739–743, https://doi.org/10.1046/j.1523-1747.1998.00361.x.
[10] D.J. Schibli, H.N. Hunter, V. Aseyev, T.D. Starner, J.M. Wiencek, P.B. McCray Jr., B.F. Tack, H.J. Vogel, The solution structures of the human beta-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against *Staphylococcus aureus*, J. Biol. Chem. 277 (2002) 8279–8289, https://doi.org/10.1074/jbc.M108830200.
[11] E.H. Mattar, H.A. Almehdar, H.A. Yacoub, V.N. Uversky, E.M. Redwan, Antimicrobial potentials and structural disorder of human and animal defensins, Cytokine Growth Factor Rev. 28 (2016) 95–111, https://doi.org/10.1016/j.cytogfr.2015.11.002.
[12] P.H. Warnke, I.N. Springer, P.A. Russo, J. Wiltfang, H. Essig, M. Kosmahl, E. Sherry, Y. Acil, Innate immunity in human bone, Bone 38 (2006) 400–408, https://doi.org/10.1016/j.bone.2005.09.003.
[13] P. Stockmann, F. Wehrhan, S. Schwarz-Furlan, F. Stelzle, S. Trabert, F.W. Neukam, E. Nkenke, Increased human defensine levels hint at an inflammatory etiology of bisphosphonate-associated osteonecrosis of the jaw: an immunohistological study, J. Transl. Med. 9 (2011) 135, https://doi.org/10.1186/1479-5876-9-135.
[14] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[15] I. Nomura, E. Goleva, M.D. Howell, Q.A. Hamid, P.Y. Ong, C.F. Hall, M.A. Darst, B. Gao, M. Boguniewicz, J.B. Travers, D.Y.M. Leung, Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes, J. Immunol. 171 (2003) 3262–3269, https://doi.org/10.4049/jimmunol.171.6.3262.
[16] U.K. Gursoy, E. Könönen, Understanding the roles of gingival beta-defensins, J. Oral Microbiol. 4 (2012) 15127, https://doi.org/10.3402/jom.v4i0.15127.
[17] M. Zirk, M. Kreppel, J. Buller, J. Pristup, F. Peters, T. Dreiseidler, M. Zinser, J. E. Zöller, The impact of surgical intervention and antibiotics on MRONJ stage II and III – retrospective study, J. Craniomaxillofac. Surg. 45 (2017) 1183–1189, https://doi.org/10.1016/j.jcms.2017.05.027.
[18] J.M. Munita, C.A. Arias, Mechanisms of antibiotic resistance, Microbiol. Spectr. 4 (2016) 481–511, https://doi.org/10.1128/microbiolspec.VMBF-0016-2015.
[19] World Health Organization, Antimicrobial Resistance: Global Report on Surveillance, 2014 (accessed 23 September 2020), http://www.who.int/drugresistance/documents/surveillancereport/en/.
[20] M. Wilmes, H.G. Sahl, Defensin-based anti-infective strategies, Int. J. Med. Microbiol. 304 (2014) 93–99, https://doi.org/10.1016/j.ijmm.2013.08.007.